



Science Press



Variability and divergence in horsegram (*Dolichos uniflorus*)

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Abstract: Horsegram is an important and unexploited tropic and sub-tropic legume crop grown mostly in dry land agriculture. The study involving 23 cultivars of horsegram (*Dolichos uniflorus*) was conducted at the Seed Research & Technology Centre in Rajendranagar, Hyderabad, India during the early spring seasons of 2008 and 2009 to assess the nature and magnitude of variability in the existing species and also to identify diverse parents for use in further breeding programmes. The results indicated significant differences among the 23 cultivars for all characters studied, indicating the presence of sufficient genetic variation. High variability and heritability coupled with high genetic advance were reported for seed yield per plant and pod hulk per plant, indicating additive gene action and a possible scope for the improvement of these characters. Mahalanobis D^2 statistics grouped all the 23 cultivars of horsegram into six clusters. The maximum inter cluster distance (62.39) was noticed between cluster IV (HG 50) and cluster V (HG 11). The maximum intra cluster distance ranged from 0 (clusters III, IV, V and VI) to 15.17 (cluster I), indicating that the genotypes in these clusters were relatively more diverse than the genotypes within other clusters. Seed yield per plant contributed the maximum (33.20%) to the genetic divergence.

Keywords: horsegram; *Dolichos uniflorus*; variability; divergence; genotype; yield components; seed quality parameters

Horsegram (*Dolichos uniflorus*) is a twining herb of Old World tropics cultivated in India for food and fodder, sometimes placed in the genus *Dolichos*. The original Latin name for horsegram was *Dolichos biflorus*, which was later changed to *D. uniflorus*. An exact synonym for horsegram is *Macrotyloma uniflorum*. The members of species *Macrotyloma uniflorum* include *Macrotyloma uniflorum* var. *stenocarpum* and *Macrotyloma uniflorum* var. *uniflorum*. Other scientific names for horsegram are *D. uniflorus*, *Dolichos uniflorus* Lam. var. *stenocarpus*, *Dolichos biflorus* and *Dolichos benadirianus*. The perennial horsegram belongs to *Macrotyloma africanum*, *Macrotyloma axillare* and *Macrotyloma bieense*.

Horsegram is native to Africa (Angola, Botswana, Democratic Republic of Congo (Zaire), Ethiopia, Kenya, Mozambique, Namibia, Rwanda, Somalia, South Africa (Transvaal), Sudan, Tanzania, Uganda and Zimbabwe), Asia (Bhutan, China, India, Indonesia (Java), Nepal, Pakistan, Philippines, Sri Lanka) and

Australia. Indian collections originate from the areas with annual rainfall from 600 to 2,200 mm, usually with high summer dominance. Horsegram needs to be cultivated in the areas with rainfall of over 300 mm. In Gujarat, Rajasthan and the Madhya Pradesh hills of western India, indigenous farmers lacking resources now have the choice of cultivating horsegram.

The whole seeds of horsegram are generally utilized as cattle feed. And the seed, sprout, or whole meal of horsegram can be used by large populations in rural areas of southern India. One of the reasons for very low productivity in horsegram is limited variability (Maloo, 1999). The selection of genetically diverse parents for hybridization programmes is most crucial for generating genetic variability in the crop. Hence, the study assessed the nature and magnitude of variability in the horsegram. The present horsegram includes released varieties (PLM 1, PLM 2 and AK 38

Received 2011-06-15; accepted 2011-09-14

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released by Achary NG Ranga Agricultural University, Hyderabad), locally cultivated varieties and a germplasm collection, representing the diverse nature of the existing horsegram cultivars. Such diverse species is properly identified for hybridization and offers the scope for generating potential genotypes that provide higher variability. The Mahalanobis D^2 technique has been very widely used to measure genetic diversity (Murthy and Arunachalam, 1966). Hence, it is proposed to use this technique to understand the nature and magnitude of genetic diversity in the existing cultivars and for further creation of more variation.

1 Materials and methods

The study comprised 23 cultivars of horsegram. Three were released varieties and the remaining 20 included locally cultivated varieties and germplasm accessions. The study was conducted at the Seed Research & Technology Centre in Rajendranagar, Hyderabad, India in the early spring seasons of 2008 and 2009. The experiment was run in three replications using a randomized block design.

Each entry is sown in two rows of 4-m length with inter spacing of 30 cm and intra spacing of 10 cm. Agronomic practices were followed to raise a healthy crop, and precautionary measures were taken for the control of pests and diseases. We recorded qualitative data (stem, leaf, pod and seed morphological characters), quantitative characters (yield and yield components) and seed quality parameters. The quantitative characters of five randomly selected plants in each treatment were observed. The seeds of all cultivars were tested for laboratory germination (paper towel) *as per* the International Seed Testing Association rules (ISTA, 1985). The final count was recorded and expressed in percentage. After the final germination count, ten normal seedlings were selected at random in each replication for recording seedling length in centimeters (cm) and the same seedlings were oven dried at 80°C for 17 h and weighed (g) for dry weights. The seedling vigour index I (SVI I) and seedling vigour index II (SVI II) were calculated by multiplying the germination percentage with seedling length and dry matter production, respectively (Abdul-Baki and Anderson, 1973).

The phenotypic and genotypic coefficients of vari-

ability (PCV, GCV) were calculated by the formulae suggested by Burton (1952). Heritability, in a broad sense, was estimated by the method described by Lush (1940) and GA as percentage of mean according to Johnson *et al.* (1955). The genetic diversity was studied using the Mahalanobis D^2 technique (Mahalanobis, 1936) and the genotypes were grouped into different clusters following Tocher's method (Rao, 1952).

2 Results and discussion

2.1 Genetic variability

Statistically analyzed data (Table 1) revealed highly significant differences with respect to the treatment mean sum of squares for all traits, indicating the presence of sufficient genetic variation among the cultivars. The variance of genotypes for all traits was significant. The general mean, genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), heritability, genetic advance (GA) and GA as percentage of mean are presented in Table 1. In the study, PCV was marginally higher than their corresponding GCV, reflecting the environmental influence on all traits and indicating that selection on a phenotypic basis will hold good for a genetic basis. Genotypic coefficient of variance (GCV) is considered to be more useful than PCV for assessing variability, as it depends on the heritable portion of variability. The difference between PCV and GCV was very high for the number of primary branches per plant, the plant height and the number of secondary branches, indicating greater environmental influence on the expression of these characters. On the other hand, seed yield per plant, pod hulk per plant and test weight showed very little difference between PCV and GCV, indicating that these characters were only slightly influenced by the environment.

The highest GCV was observed for pod hulk per plant (42.26 g), followed by pods per plant (34.44 g) and seed yield per plant (34.10 g). Samal and Senapati (1997) reported the highest PCV and GCV for seed yield per plant in horsegram. On the other hand, low GCV estimates were noticed for the germination rate (1.21%), seedling length (4.03 cm) and seedling vigour index I (4.41), indicating a narrow range of variability for these characters and restricting the scope for

Table 1 Variability for different characters in horsegram

Character	Mean	Genotypic variance	Phenotypic variance	Genotypic coefficient of variance (%)	Phenotypic coefficient of variance (%)	Heritability (%)	Genetic Advance (GA)	GA as percentage of mean (%)
Leaf length (cm)	5.26	0.55	0.62	14.08	15.00	88.1	1.43	27.23
Leaf width (cm)	3.30	0.44	0.48	20.12	21.06	91.3	1.31	39.61
Plant height (cm)	53.51	16.88	88.62	7.679	17.59	19.1	3.70	6.90
Number of primary branches	10.11	0.43	3.40	6.45	18.23	12.5	0.48	4.70
Number of secondary branches	8.08	3.97	6.91	24.66	32.54	57.4	3.11	38.49
Number of pods per axil	3.38	0.19	0.34	12.72	17.34	53.8	0.65	19.23
Number of pods per plant	79.34	746.78	1,047.27	34.44	40.79	71.3	47.54	59.91
Number of seeds per pod	5.65	0.27	0.33	9.10	10.23	79.2	0.94	16.69
Pod length (cm)	5.59	0.10	0.16	5.72	7.12	64.5	0.53	9.46
Pod hulm per plant (g)	6.29	7.04	7.20	42.26	42.75	97.7	5.40	86.06
Seed yield per plant (g)	13.00	19.66	20.02	34.10	34.41	98.2	9.05	69.60
Test weight (g)	3.22	0.09	0.17	9.37	10.14	85.4	0.57	17.83
Germination rate (%)	98.37	1.41	5.42	1.21	2.37	26.0	1.25	1.27
Seedling length (cm)	25.86	1.09	5.33	4.03	8.93	20.4	0.97	3.74
Seedling dry weight (g)	0.02	0.00	0.00	10.68	13.21	65.3	0.00	17.78
Seedling vigour index I	2,542.89	12,556.64	57,833.16	4.41	9.46	21.7	107.56	4.23
Seedling vigour index II	2.16	0.05	0.07	10.11	12.42	66.3	0.37	16.96

selection. Samal and Senapati (1997) in horsegram, Singh (1994) in French bean and Samal (1996) in rajmash reported high disparities between GCV and PCV for the number of plant branches, but low GCV and PCV for days to 50% flowering.

Seed yield per plant exhibited the highest heritability (98.2%), followed by the pod hulm per plant (97.7%) and leaf width (91.3%). The selection for these characters is likely to be effective. The other characters noticed for high heritability estimates were leaf length (88.1%), test weight (85.4%), the number of seeds per pod (79.2%) and the number of pods per plant (71.3%), respectively. Samal and Senapati (1997) reported high heritability estimates for the number of pods per plant, seeds per pod and seed yield per plant.

On the other hand, the number of primary branches per plant (12.5%), plant height (19.1%) and seedling length (20.4%) exhibited the lowest heritability; selection for such traits is ineffective. Dobhal and Rana (1994) reported high heritability for clusters per plant, the number of pods per plant, seed yield per plot and low heritability for branches per plant.

Heritability estimates, along with GA as percentage of mean, are more important for improvement rather than heritability alone (Johnson *et al.*, 1955). GA as percentage of mean was high for the number of pod hulm per plant (86.06%), seed yield per plant (69.60%)

and the number of pods per plant (59.91%). These results indicate that these characters are controlled by additive gene action; phenotypic selection for the improvement of these characters may be effective. In term of horsegram, Venkateswarlu (2000) reported high GA as percentage of mean for clusters per plant and the number of pods per plant. Dobhal and Rana (1994) also reported that clusters per plant could be improved through phenotypic selection. Low GA as percentage of mean was noticed for seed quality parameters such as the germination rate (1.27%), seedling length (3.74 cm) and SVI I (4.23). In the present study, high heritability coupled with a high GCV value was recorded for seed yield per plant and the pod hulm per plant, suggesting the possibility for improvement of these characters. Samal and Senapati (1997) reported high heritability coupled with high genetic advance for yield per plant, the numbers of pods per plant and seeds per pod in horsegram.

High variability and heritability coupled with high genetic advance were reported for seed yield per plant and pod hulm per plant, indicating additive gene action. Thus there is greater scope for the selection of these characters. Leaf width and length recorded high heritability coupled with moderate genetic advance and the scope for their improvement was restricted by the low range of variability present in the population.

2.2 Genetic divergence

Mahalanobis D^2 statistics grouped all the 23 cultivars of horsegram into six clusters (Table 2 and Fig. 1). The cluster's strength varied from single/solitary genotypes to fourteen genotypes. Cluster I had fourteen cultivars, cluster II had five cultivars and the other four clusters (III, IV, V and VI) had a single genotype each (Table 2). The distribution pattern of genotypes into six clusters confirmed the existence of diversity among the genotypes, as also indicated by analysis of variation (Mittal and Brar, 2008). The average intra and inter cluster distances are given in Table 3. The inter cluster distance between cluster IV (HG 50) and cluster V (HG 11) was the maximum (62.39). The characteristic of cluster V was that it showed a considerably higher mean value for leaf width (4.09 cm), the numbers of primary branches per plant (12) and secondary branches per plant (11.1), seed yield per plant (21.98 g), test weight (3.56 g) and seedling length (27.38 cm). Hence, crossing between the genotypes grouped in clusters IV and V may lead

to the realization of a wide spectrum of variability and transgressive segregants for the aforementioned traits. In turn, the selection for high-yielding horsegram genotypes can be made. The intra cluster distance ranged from 0 (clusters III, IV, V and VI) to 15.17 (cluster I). The maximum intra cluster distance in cluster I indicated that genotypes in this cluster were relatively more diverse than the genotypes within other clusters. The other high inter cluster distances were noticed between clusters IV and VI (61.20), clusters III and VI (59.95), and clusters III and V (57.72). This indicates that crossing between the genotypes of such clusters may be rewarding.

The minimum distance between clusters III and IV indicated that they are genetically close. The observed distances reflected the genetic diversity in the cultivars and their linkage with respect to one another (Katiyar *et al.*, 2009). The selection of parents from the two clusters may be avoided, as it represents a narrow genetic base. Further selection of parents for crossing programmes should be done from two clusters having

Table 2 Group constellation of twenty-three genotypes of horsegram based on D^2 values

Clusters	Number of genotypes	Genotypes
Cluster I	14	HG 63, HG 15, HG 41, HG 52, HG 58, HG 75, PALEM 1, HG 18, HG 72, HG 46, HG 59, HG 49, HG 54, HG 35
Cluster II	5	HG 14, HG 32, HG 38, HG 24, PALEM 2
Cluster III	1	HG 17
Cluster IV	1	HG 50
Cluster V	1	HG 11
Cluster VI	1	AK 38

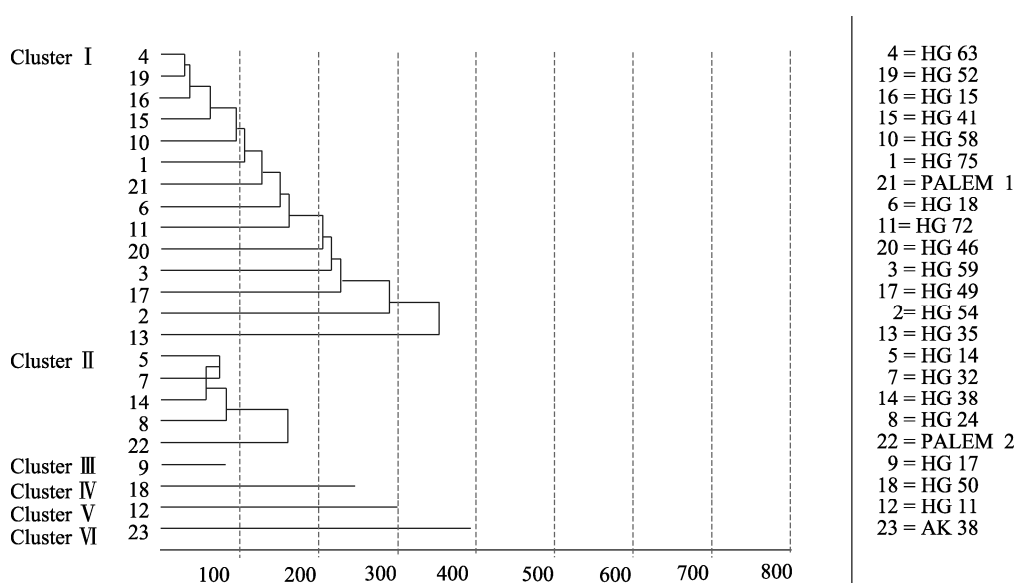


Fig. 1 Clustering of horsegram genotypes by Tocher Method (Rao, 1952)

Table 3 Intra (diagonal) and inter cluster divergence for six clusters in horsegram

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	15.17	25.51	21.00	23.14	43.93	44.89
Cluster II		11.81	37.72	42.57	23.74	28.20
Cluster III			0.00	18.18	57.72	59.95
Cluster IV				0.00	62.39	61.20
Cluster V					0.00	19.82
Cluster VI						0.00

wider inter cluster distances to get maximum variability. In the present study, different clusters showed superiority for different traits. Knowledge on the contribution of these traits to genetic diversity is also essential so that the parents can be selected for hybridization programmes. It was stated that the characters contributing the maximum to the divergence should be given greater emphasis for deciding on the clusters for the purpose of further selection and choice of parents

for hybridization.

The cluster means for different characters, along with the relative contribution of different characters to the expression of genetic divergence (Table 4), showed that seed yield per plant (33.20%) contributed the maximum to the genetic divergence, followed by the seedling vigour index I (27.27%), test weight (10.28%), seedling vigour index II (9.49%) and pod hulk per plant (7.11%). It was reported that plant height and seed yield per plant were considered to be the most important characters contributing to genetic divergence in cowpea. On the other hand, Ushakumari *et al.* (2000) grouped 50 cowpea genotypes into thirteen clusters and reported that among the yield contributing characters, number of seeds per pod, branches per plant, pods per cluster and pod length were the important traits responsible for the divergence recorded.

Table 4 Cluster means and contribution rate of seed yield, yield component and seed quality characters in horsegram

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Contribution rate (%)
Leaf length (cm)	5.27	4.96	4.73	5.06	6.03	6.65	0.79
Leaf width (cm)	3.36	3.16	2.19	3.43	4.09	3.40	0.79
Plant height (cm)	51.36	57.17	45.90	48.51	65.43	65.99	0.00
Number of primary branches	9.69	11.13	11.10	7.70	12.00	10.40	0.00
Number of secondary branches	8.35	7.13	6.70	4.75	11.10	10.70	0.00
Number of pods per axil	3.43	3.38	2.86	3.10	3.26	3.60	0.00
Number of pods per plant	76.91	86.39	45.00	41.95	73.30	156.00	0.40
Number of seeds per pod	5.76	5.79	5.80	5.20	5.50	3.90	1.58
Pod length (cm)	5.70	5.45	5.42	5.27	5.23	5.63	1.19
Pod hulk per plant (g)	5.39	7.38	3.34	2.55	12.30	13.88	7.11
Seed yield per plant (g)	11.41	16.64	7.13	6.03	21.98	20.96	33.20
Test weight (g)	3.16	3.44	3.41	2.37	3.56	3.13	10.28
Germination rate (%)	98.49	97.58	99.50	97.50	99.15	99.65	0.79
Seedling length (cm)	25.98	25.84	27.32	21.07	27.38	26.15	4.35
Seedling dry weight (g)	0.02	0.02	0.02	0.03	0.02	0.02	2.77
Seedling vigour index I	2,557.93	2,516.46	2,717.45	2,055.60	2,716.05	2,604.20	27.27
Seedling vigour index II	2.16	2.15	1.88	2.46	2.13	2.19	9.49

Table 5 Desirable characters selected from diverse clusters

Cluster	Desirable characters and mean values in parentheses
I	Pod length (5.70 cm)
II	Nil
III	Number of seeds per pod (5.80); Seedling vigour index I (2,717.45)
IV	Seedling dry weight (0.03 g); Seedling vigour index II (2.46)
V	Leaf width (4.09 cm); Number of primary branches per plant (12.00); Number of secondary branches per plant (11.10); Seed yield per plant (21.98 g); Test weight (3.56 g); Seedling length (27.38 cm)
VI	Leaf length (6.65 cm); Plant height (65.99 cm); Number of pods per axil (3.60); Number of pods per plant (156.00); Pod hulk per plant (13.88 g); Germination rate (99.65%)

Among the different yield components, yield and seed quality parameters showed the maximum divergence; cluster I had the maximum pod length (5.70 cm), and cluster III had the maximum number of seeds per pod (5.80) and SVI I (2717.45); cluster IV had the maximum seedling dry weight (0.03 g) and SVI II (2.46); cluster V had the maximum leaf width (4.09 cm), the number of primary branches per plant (12.00), the number of secondary branches per plant (11.10), seed yield per plant (21.98 g), test weight (3.56 g) and seedling length (27.38 cm); and cluster

VI had the maximum leaf length (6.65 cm), plant height (65.99 cm), the number of pods per axil (3.60), the number of pods per plant (156.00), pod hulk per plant (13.88 g) and germination rate (99.65%) (Table 5).

Thus, it can be concluded that selection of genotypes from the most divergent clusters may exhibit a high heterosis besides grain yield. Therefore, hybridization between the genetically diverse parents in further breeding programmes may produce large variability and better recombinants in the segregating generations.

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